

TRH-Stimulation of Prolactin Release from Bovine Pituitary Cells¹ (38745)

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Synthetic thyrotropin releasing hormone (TRH) causes release of thyroid stimulating hormone (TSH) from pituitaries of several species including man (1), rat (2, 3) and bovine (4). In addition, prolactin production was increased and growth hormone production decreased by cloned rat pituitary tumor cells exposed to TRH (5). But TRH was not shown to consistently cause release of prolactin from normal rat hemipituitaries (6, 7) or bovine pituitary explants (4, 8) *in vitro*. However, TRH will increase serum prolactin concentration in humans (9, 10), rat (11), bovine (8, 14) and sheep (12) and serum growth hormone concentration in cattle (8) and acromegalic humans (15). The objective of the present investigation was to use dispersed bovine pituitary cells to re-examine the effect of TRH on prolactin and growth hormone release *in vitro*.

Materials and Methods. Bovine pituitaries (cows, steers and a bull) were collected at a local abattoir within 30 min of killing and transported at 37° to the laboratory. Within 1 hr of death of the animals, posterior pituitaries were discarded, anterior pituitaries were minced and cell dispersion procedures initiated as previously described (16). Briefly, anterior pituitaries were dispersed using 0.2–0.3% collagenase at 37° for 1 hr after which the dispersed cells were passed through cheesecloth, washed four times and resuspended in 120 ml TC medium 199: Eagle's minimal essential medium—Hank's base 50:50 (v/v) containing 10% cow serum. Four ml of this cell suspension were transferred to 30 ml tissue culture flasks (Falcon Plastics, Oxnard, CA) and incubated at 37° for 3–4 days. During this period medium was replaced at 24 hr intervals beginning at 48 hr. On the day of the experiment the medium used for growing the cells was replaced with TC Medium 199 (without cow serum) and

cells were incubated for 2 hr. The medium was decanted and stored frozen until assayed for hormone concentration. Varying concentrations of synthetic TRH² in 4 ml TC Medium 199 were added to the flasks (4 flasks/treatment) and incubation continued for an additional 2 hr. Pituitary cells from four cows were in culture 72 hr when they were exposed to 0.0, 0.01, 0.1, or 10.0 ng TRH/ml medium. In a second experiment pituitary cells from four cows and three steers were in culture for 96 hr when they were incubated with 0.0, 0.01, 0.1, 1.0, 10.0 or 100.0 ng TRH/ml medium. In addition pituitary cells from a bull were incubated with 0.0, 10.0 or 100.0 ng TRH/ml medium at 96 hr of culture. Following incubation with TRH the media were decanted and frozen for hormone assay. Media prolactin and growth hormone concentrations were determined by specific radioimmunoassay as reported from our laboratory (17, 18).

Results. Addition of TRH to 72-hr pituitary cell culture from cows increased ($P < 0.01$) prolactin release into the media two- to fivefold relative to that for the 2 hr pretreatment period (Table I) Prolactin concentration averaged approximately 0.5 $\mu\text{g/ml}$ for all treatment groups during the 2 hr pretreatment period. Following 2 hr of incubation with 0 ng TRH/ml media average prolactin concentration was decreased 4% ($P > 0.05$) relative to comparable prolactin values for the 2 hr pretreatment period. However, TRH increased prolactin release 136, 351, 351, and 389% when added to the media at concentrations of 0.01, 0.1, 1.0 and 10.0 ng/ml TRH, respectively. Apparently, maximum prolactin release was achieved with 0.1 ng TRH/ml medium in this experiment since magnitude of prolactin release among the three highest doses of TRH tested in this experiment was not significant.

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TABLE I. PROLACTIN RELEASE FROM BOVINE PITUITARY CELL CULTURES TREATED WITH TRH.^a

TRH	Prolactin in media		Δ^c
	Pretreatment ^b	Posttreatment	
(ng/ml)	(ng/ml)		
0	576 ± 39	553 ± 79	-23 ± 78 ^d
0.01	587 ± 38	1386 ± 204	799 ± 174 ^e
0.1	560 ± 52	2526 ± 59	1966 ± 42 ^f
1.0	548 ± 6	2474 ± 116	1926 ± 120 ^f
10.0	508 ± 51	2484 ± 157	1976 ± 117 ^f

^a Values are means ± standard error.

^b Mean prolactin concentration of four flasks for the 2-hr period preceding treatment.

^c Difference between pre- and posttreatment.

^{d, e, f} Δ values with different superscripts are different ($P < .05$).

TABLE II. GH RELEASE FROM BOVINE PITUITARY CELL CULTURES TREATED WITH TRH.^a

TRH	GH in media		Δ^c
	Pretreatment	Posttreatment	
(ng/ml)	(ng/ml)		
0	90 ± 3	85 ± 4	-5 ± 3 ^d
0.01	111 ± 12	120 ± 7	9 ± 6 ^e
0.1	102 ± 11	114 ± 9	12 ± 4 ^e
1.0	109 ± 7	130 ± 5	21 ± 4 ^f
10.0	101 ± 4	122 ± 8	21 ± 5 ^f

^a Values are means ± standard error.

^b Mean GH concentration of four flasks for the 2-hr period preceding treatment.

^c Difference between pre- and posttreatment.

^{d, e, f} Δ values with different superscripts are different ($P < .05$).

Growth hormone concentrations in these same media are shown in Table II. Although all doses of TRH increased ($P < 0.05$) growth hormone release relative to controls, the magnitude of response was small compared to that of prolactin. Addition of TRH at levels as low as 0.01 ng/ml media increased growth hormone concentrations as evidenced from the mean differences in growth hormone concentration between the 2 hr pre- and posttreatment periods. Maximum growth hormone release was attained with a concentration of 1.0 ng/ml media.

To determine whether the effects of TRH

TABLE III. PROLACTIN RELEASE FROM BOVINE PITUITARY CELL CULTURES TREATED WITH TRH.^a

TRH	Prolactin in media		Δ^c
	Pretreatment ^b	Posttreatment	
(ng/ml)	(ng/ml)		
0	173 ± 7	146 ± 9	-27 ± 14 ^d
0.01	167 ± 21	230 ± 23	63 ± 3 ^e
0.1	193 ± 23	302 ± 42	109 ± 20 ^f
1.0	201 ± 9	408 ± 21	207 ± 29 ^g
10.0	258 ± 15	484 ± 42	226 ± 27 ^g
100.0	230 ± 21	367 ± 37	137 ± 20 ^f

^a Values are means ± standard error; 96-hr pituitary cell cultures.

^b Mean prolactin concentration of four flasks for the 2-hr period preceding treatment.

^c Difference between pre- and posttreatment.

^{d, e, f, g} Δ values with different superscripts are different ($P < .05$).

on prolactin and growth hormone release from cow pituitary cells was repeatable and to extend our observations to pituitary cells from steers and bulls, a second experiment was conducted. Addition of TRH to 96-hr pituitary cell cultures from cows, steers and a bull increased ($P < 0.05$) prolactin release into the media (Tables III, IV). Similar to the 72-hr cell culture there was a decrease in media prolactin concentration from control flasks for cows, steers and the bull during the second 2-hr incubation period relative to the pretreatment period. Magnitude of prolactin release from cells of cow pituitaries in this experiment averaged $\approx 37\%$ that of cow pituitary cell cultures in the first experiment. But, 0.01 ng TRH/ml medium still evoked prolactin release from these cells (Table III). However, in the second experiment maximum prolactin release was not achieved until 1.0 ng TRH/ml was included in the medium (Table III) and extending the dose of TRH to 100 ng/ml medium caused a reduction in prolactin release relative to that evoked by 10 ng TRH.

TRH also augmented release of prolactin from steer pituitary cell cultures (Table IV). Prolactin concentration in flasks receiving 0.1 ng TRH/ml media was increased ($P < 0.05$) relative to control flasks. It appeared that addition of 10 ng TRH/ml medium

TABLE IV. PROLACTIN RELEASE FROM BOVINE PITUITARY CELL CULTURES TREATED WITH TRH^a.

TRH (ng/ml)	Prolactin in media		Δ^c	Sex (n)
	Pretreatment ^b	Posttreatment		
0	123 ± 21	109 ± 12	-14 ± 10 ^d	Steer ()
0.01	110 ± 9	123 ± 5	13 ± 13 ^d	
0.1	140 ± 4	176 ± 11	36 ± 8 ^e	
1.0	140 ± 14	293 ± 26	153 ± 30 ^f	
10.0	109 ± 9	275 ± 7	166 ± 6 ^f	
100.0	112 ± 8	192 ± 11	80 ± 10 ^g	Bull (1)
0	57 ± 4	51 ± 5	-6 ± 6	
10.0	64 ± 8	78 ± 10	14 ± 6	
100.0	72 ± 6	103 ± 18	31 ± 12	

^a Values are means ± standard error; 96-hr pituitary cell cultures.

^b Mean prolactin concentration of four flasks for the 2-hr period preceding treatment.

^c Difference between pre- and posttreatment.

^{d, e, f, g} Δ values with different superscripts are different ($P < .05$).

caused no further increase in prolactin release relative to 1.0 ng TRH/ml medium. But in flasks receiving 100 ng TRH/ml medium, there was a decrease ($P < 0.05$) in prolactin release relative to the response at 10 ng/ml.

Addition of TRH to pituitary cell cultures from a bull increased prolactin release into the media (Table IV). Unlike results attained with cells from cows and steers, there was no decrease in magnitude of prolactin release by 100 ng/ml TRH compared to values at 10 ng/ml. In the second experiment media growth hormone concentration (ng/ml) during the second incubation period was less than comparable values during the pre-treatment period (Tables V, VI). This pattern was observed among cow, steer or bull pituitary cell cultures and was independent of dose of TRH used. We believe that this decrease in growth hormone concentration was not due to TRH inhibition since analysis of variance revealed that magnitude of decrease among TRH-treated flasks was not different from control flasks ($P > .05$).

Discussion. Results reported here clearly demonstrated stimulation of prolactin release from bovine pituitary cells in culture by synthetic TRH. Vale *et al.* (19) reported enhanced prolactin secretion by pituitary cells, from normal and propylthiouracil fed rats, which were exposed to TRH at concentrations of 10^{-9} – 10^{-6} M. In the present investigation prolactin release from bo-

TABLE V. GROWTH HORMONE RELEASE FROM BOVINE PITUITARY CELL CULTURES TREATED WITH TRH^a.

TRH (ng/ml)	Growth hormone in media		Δ^c
	Pretreatment	Posttreatment	
0	104 ± 6	58 ± 11	-46 ± 16
0.01	108 ± 12	71 ± 12	-37 ± 2
0.1	103 ± 15	53 ± 11	-50 ± 5
1.0	112 ± 3	60 ± 5	-52 ± 7
10.0	146 ± 21	89 ± 10	-57 ± 16
100.0	115 ± 12	51 ± 8	-64 ± 5

^a Values are means ± standard error; 96-hr pituitary cell cultures from cows.

^b Mean GH concentration of four flasks for the 2-hr period preceding treatment.

^c Difference between pre- and posttreatment.

vine pituitary cells was increased by TRH at concentrations as low as 3×10^{-11} M. The decrease in magnitude of prolactin release in response to TRH at high concentrations (3×10^{-7} M) has not, to our knowledge, been reported previously. The reason for this decrease in prolactin response is not known but perhaps the tripeptide assumes a degree of toxicity at higher concentrations.

We previously reported no stimulation of prolactin release from steer pituitary explants *in vitro* (8) and other investigators (4)

TABLE VI. GROWTH HORMONE RELEASE FROM BOVINE PITUITARY CELL CULTURE TREATED WITH TRH^a.

TRH (ng/ml)	Growth hormone in media		Δ^c	Sex (n)
	Pretreatment ^b	Posttreatment		
0	96 ± 10	58 ± 5	38 ± 8	Steer (3)
0.01	100 ± 12	69 ± 10	-31 ± 11	
0.1	123 ± 7	60 ± 7	-63 ± 3	
1.0	130 ± 12	79 ± 8	-51 ± 16	
10.0	100 ± 12	80 ± 7	-20 ± 12	
100.0	106 ± 2	60 ± 7	-46 ± 6	
0	55 ± 7	41 ± 5	-14 ± 5	Bull (1)
10.0	68 ± 7	47 ± 8	-21 ± 6	
100.0	77 ± 7	40 ± 8	-37 ± 2	

^a Value are means ± standard error; 96-hr pituitary cell culture.

^b Mean GH concentration of four flasks for the 2-hr period preceding treatment.

^c Difference between pre- and posttreatment.

reported a marginal increase in prolactin release in one of three experiments with bovine pituitary explants *in vitro*. Thus, TRH repeatedly causes prolactin release from bovine pituitary cells in culture but prolactin release from bovine pituitary explants is not demonstrable or at best variable. Perhaps enzymatic dispersion of pituitary cells causes cellular transformation that results in release of prolactin by TRH from these cells but not from explants. But this view is not supported by Vale *et al.* (19) who reported TRH-induced prolactin release by dispersed rat pituitary cells and by incubated rat hemipituitaries. Another possible explanation involves nonspecific release of the hormones from freshly prepared explants. Because of the size of the bovine pituitary, excessive cutting of the tissue is required to achieve explants of desirable size (compare this to simply bisecting the rat pituitary). As a result, cell destruction must occur. Although preincubation of explants is a usual part of these procedures it is possible that part of the quantity of hormone found in the media is the result of diffusion and part from emiocytosis of secretory granules. Obviously only the latter portion of hormone release can respond to a secretagogue such as TRH. If passive release is much larger than specific release of hormone then it is possible that a stimulatory effect of a secretagogue could be masked by the relatively large mass of hormone being passively released. Holding

pituitary cells in culture should maximize the proportion of functional cells since dead or damaged cells would be eliminated in media changes during the growth period. Accordingly, the response of these functional cells should be clearly defined in the absence of a high background release of hormone.

Thyrotropin releasing hormone consistently causes increased serum growth hormone in cows (8) and has been reported to increase serum growth hormone concentration in acromegalic patients (15). But the effect of TRH on growth hormone release *in vitro* is equivocal. Tashjian *et al.* (5) reported a decrease in growth hormone release by the same tumor cells that secreted increased quantities of prolactin in response to TRH. Similarly, addition of TRH to normal rat hemipituitaries *in vitro* did not change growth hormone release (6). In contrast, Labella and Vivian (4) reported enhanced growth hormone release in response to TRH in one of three experiments with bovine pituitary explants and in a preliminary report, TRH was shown to increase growth hormone secretion by primary cell cultures of calf pituitary (20). Results of the present experiment did not clarify the effect of TRH on growth hormone release *in vitro*. The reason why TRH caused growth hormone release in the first experiment but not the second is not clear.

Summary. Bovine anterior pituitaries were dispersed with collagenase and the cells

grown to monolayers. On day 3 or 4 of culture, cells (4 flasks/treatment group) were incubated for 2 hr with TC medium 199, then with TC medium 199 containing thyrotropin releasing hormone (TRH) for 2 hr. Relative to values during the first 2-hr incubation, prolactin concentration in media from cow pituitary cell cultures exposed to 0 (control), 0.01, 0.1, 1.0 or 10.0 ng TRH/ml medium averaged -23, 799, 1966, 1926 and 1976 ng/ml. Relative to control, each dose of TRH increased ($P < 0.01$) media prolactin concentration. Comparable values for growth hormone were -5, 9, 12, 21, and 21 ng/ml and the increase in growth hormone release relative to controls was significant ($P < 0.05$). A second experiment, designed to determine whether TRH would increase prolactin release by pituitary cells from cows, steers and a bull was conducted using the same procedures. Change in media prolactin concentration (ng/ml) after 0, 0.01, 0.1, 1.0, 10.0 and 100.0 ng TRH/ml medium was -27, 63, 109, 207, 226 and 137 for cows and -14, 13, 36, 153, 166 and 80 for steers. After 0.0, 10 and 100 ng TRH/ml medium comparable values for a bull were -6, 14 and 31 ng/ml. Growth hormone (ng/ml) in these media was not different from controls. We conclude that TRH stimulates prolactin release from pituitary cells of cows, steers and bulls but its effect on growth hormone release is not consistent.

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